IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Remacle et al.
Appl. No.	:	09/817,014
Filed	:	March 23, 2001
For	:	IDENTIFICATION OF BIOLOGICAL (MICRO)ORGANISMS BY DETECTION OF THEIR HOMOLOGOUS NUCLEOTIDE SEQUENCES ON ARRAYS
Examiner	:	Calamita, Heather
Group Art Unit	:	1637

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES TO OVERCOME CITED PUBLICATION UNDER 37 CFR 1.131

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. The declaration is to establish completion of the invention of this application in a WTO country at a date prior to the date that appears on Anthony et al. *J. Clin. Microbiol.* 2000, 38:781-788, attached, which we understand was cited by the Examiner. The above-identified application claims the benefit of European Application No.: 00870055.1 filed on March 24, 2000. Anthony et al. reference was published in February 2000. Anthony's paper describes detecting various bacterial sequences amplified using universal PCR primers and then hybridizing the obtained PCR products to capture molecules of no more than 30 nucleotides in length cross-linked to nylon strips using UV light. The identification of a specific bacterial strain was obtained by analyzing a pattern of spots on the nylon strip. The claimed invention encompasses a method of identifying homologous sequences in a sample by amplifying the sequences with a primer pair capable of amplifying at least 2 of the homologous sequences and then hybridizing the PCR products on capture molecules covalently bound to a solid support of a

biochip, wherein the capture molecules comprise a spacer of at least 40 bases in length and a specific sequence of about 15 to 40 bases in length each capable of specifically binding to only one of the target molecules.

2. The persons making this declaration are the named co-inventors.

3. To establish the date of completion of the invention of this application, the following true copies and their translations are submitted as evidence: Exhibit A: letter of February 1, 2000 by the lead inventor, Jose Remacle, to the Belgian Patent Office of the Industrial Property Department of the Ministry of Economic Affairs in Brussels; Exhibit B: letter of February 4, 2000 by the lead inventor, Jose Remacle, to Mr. Frederic Luizi of the Facultes Universitaires Notre-Dame de la Paix; Exhibit C: letter of February 24, 2000 by the lead inventor, Jose Remacle, to Office Van Malderen law firm regarding filing an application; Exhibit D: letter of February 24, 2000 by the lead inventor, Jose Remacle, to Mr. Frederic Luizi of the Facultes Universitaires Notre-Dame de la Paix; and Exhibit E: letter of February 29, 2000 by the lead inventor, Jose Remacle, to Mr. Frederic Luizi of the Facultes Universitaires Notre-Dame de la Paix.

4. Exhibit A shows that as of February 1 2000, Dr. Jose Remacle was asking Mr. Lambermont of the Belgian Patent Office of the Industrial Property Department of the Ministry of Economic Affairs to conduct a preliminary search for a new invention for simultaneous assay for homologous DNA sequences present in a biological sample by hybridization on a surface containing various capture probes having a specific sequence between 10 and 60 nucleotides and a total length between 40 and 400 nucleotides.

5. Exhibit B shows that as of February 4, 2000, Dr. Jose Remacle was informing Mr. Frederic Luizi of the Facultes Universitaires Notre-Dame de la Paix (former applicant and Assignee of the present application) about the content of research obtained with funds from the Walloon Region, and requesting the funds for filing of a patent application to a method and kit for the detection and/or quantification of homologous nucleic acid sequences on arrays.

6. Exhibit C shows that as of February 24, 2000 Dr. Jose Remacle was requesting that the patent attorney, Eric Van Malderen file a new patent application, the draft of which was attached to the letter. The application was to a method of detection of homologous sequences in

a sample after amplification by the same primers, and using a spacer of a specific length on a biochip.

7. Exhibit D shows that as of February 24, 2000 Dr. Jose Remacle was again writing to Mr. Frederic Luizi of the Facultes Universitaires Notre-Dame de la Paix (former applicant and Assignee of the present application) regarding filing a patent application for the new invention "Method and kit for detection and/or quantification of homologous nucleic acid sequences on arrays", wherein a single pair of primers is used for the amplification of homologous sequences and wherein single-stranded capture molecules 30-600 nucleotides in length with a part of 10-60 bases in length specific for a target molecule are fixed upon a solid support. The letter mentions that the draft of the application was already being reviewed by the patent attorney and that the search performed in the Brussels Industrial Property Department identified the invention as being novel.

8. Exhibit E shows that as of February 29, 2000 Dr. Jose Remacle was again writing to Mr. Frederic Luizi of the Facultes Universitaires Notre-Dame de la Paix (former applicant and Assignee of the present application) regarding urgency in filing the patent application for the new invention "Method and kit for detection and/or quantification of homologous nucleic acid sequences on arrays".

9. The Evidence establishes completion of the claimed invention as of February 2000.

I declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 19/9/05 By: _____
Jose Remacle

Dated: 19/9/05 By: _____
Sandrine Hennels

Appl. No. : 09/817,014
Filed : March 23, 2001

Dated: 17/09 By: _____
Nathalie Zammatteo

Dated: 17/09/2005 By: _____
Laurence Lockman

Dated: 19/09/2005 By: _____
Sophie Dufour

Dated: 19-9-2005 By: _____
Isabelle Alexandre

Dated: 19/09/2005 By: _____
Françoise De Longueville

1855752
080905

INDUSTRIAL PROPERTY DEPARTMENT
MINISTERY OF ECONOMIC AFFAIRS
Boulevard Emile Jacqmain
B-1000 BRUXELLES

Attn: Mr Lambermont

Brussels, February 1, 2000

Dear Mr Lambermont,

Would it be possible to perform a preliminary search for a new invention we have conceived.

We ask you to complete a search.

The following key words would be:

- Chips or array,
- DNA or probe,
- Capture probe length,
- Homologous sequences.

Claim 1 would be "simultaneous assay for homologous DNA sequence present in a biological sample by hybridization on a surface containing various capture probe (array), the capture probe having a specific sequence between 10 and 60 and a total length between 40 and 400."

Thank you in advance,

[signature]

José REMACLE

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Namur, le 04 février 2000

Cher Frédéric,

Comme je te l'ai signalé, nous avons un brevet à déposé sur le projet des biochips . Il s 'agit de compléter les travaux antérieurs sur les stratégies de design des sondes pour l'aplication diagnotic des biochips. Le titre du brevet serait «METHOD AND KIT FOR DETECTION AND/OR QUANTIFICATION OF HOMOLOGOUS NUCLEIC ACID SEQUENCES ON ARRAYS» . Comme c'est urgent, j'aimerais savoir si je procède comme auparavant , c'est à dire en annonçant cette demande à la DGTRE et en te transmettant une copie.

Peux-tu me signaler si c'est toujours bon

Sincèrement

José Remacle

LETTER FROM JOSE REMACLE TO FREDERIC LUIZI

Namur, the 4th of February 2000

Dear Frédéric,

As mentioned, we have to file a patent upon the biochips project.

It aims to complete previous works upon the strategies of probe design for a diagnostic application of biochips.

The title of the patent will be "method and kit for the detection and/or quantification of homologous nucleic acid sequence on arrays".

As it is urgent, I would like to know if I have to proceed as before, which means by presenting this request to DGTRE and by transmitting a copy of this request to you.

Please inform me if it is correct.

Sincerely,

José Remacle

OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUXELLES

Attn: Mr Eric VAN MALDEREN

BY FAX

Namur, February 24, 2000

Dear Eric,

Enclosed, you will find a patent relating to chips, to be precise, the chips we will develop for the detection of bacteria. We have made a considerable effort on the subject and it is working very well; the patent is thus very important to us, as this will be our working tool for other bacterial chips. I have included many examples. You will see that the patent is very similar to the one relating to transcription factors.

You will note that the results are brilliant. I wish to file a patent application soon, with the certainty, however, that a patent will indeed be issued.

The main problem I experienced is the following. You will see that the idea is to increase the size of the probes by a non-specific portion as well as their concentrations. The preliminary search has not provided any relevant prior art, but I am sure that by looking through the scientific literature in greater detail, we would find that somebody else has used probes that are similar to the ones we have developed. It is, however, very difficult to identify such prior art. In order to avoid such problems, I have limited the patent to the detection of homologous sequences after an amplification by the same primers. This includes a protection for the bacterial species diagnostics.

It is obvious that the patent's scope would be considerably broader if it would apply to the detection of all sequences, whether the latter would be homologous or not. In fact, this goes for all kinds of detection. Could you please check what we should do.

I have probably written the patent in a too scientific way, but we can, of course, change it by suppressing several paragraphs. It is easier to suppress than to add paragraphs. Similarly, I have provided many results, which show the importance of the patent. We could, however, eliminate some of these results.

You will see that this text includes several claims related to interesting products. These claims may become the first claims mentioned.

I would like you to read the text and to give us some feedback about your reaction.

OF COURSE, I DO HAVE QUESTIONS.

As to the figures, we have presented the arrays, drafts and tables. You can choose which one you consider to be best.

Is the originality clear? Should we justify everything, as is done in the patent.

For instance, we have inserted two drawings (3 and 4), thus showing that it did not work before; is this necessary? Similarly, the two drawings relating to spacers and the concentrations can be eliminated (Fig. 5 and 6).

Are the values justifying the concentrations on the support presented clearly. Concerning the examples, I have again avoided inserting the composition of the hybridization solution. We have discovered that the use of high concentrations of a detergent provides us with a better signal. This is due to the wettability of the glass support, which is not sufficient. We do not mention these aspects. We prefer to keep it secret. We did only cite the detergents in the text. Is this a good idea? In scientific literature, people do use them, but not in such high concentrations as we do.

As to the claims, there is a problem regarding the distances of the spacer. We can use nucleotides as we did, but we could also other chemical molecules. How should we protect ourselves? I have used both. The chemical molecules are defined by their distances. The problem is that it is always difficult to know the exact distance of a molecule. We could, therefore, refer to the number of atoms.

I would like to protect the chips' design with its various spots as such. Is it possible or is a 'registered trademark' required here?

The patent will be paid for by the Faculté, WHO WILL BE THE APPLICANT.

The inventors will be José REMACLE, Nathalie Zammatteo, Sandrine Hamels, Laurence Lockman, Sophie Dufour, Françoise Delongueville et Isabelle Alexandre.

I suggest that you work on these patents, that we then meet so as to take a closer look and come to an agreement concerning the claims.

I suppose that you consider both patents as not too urgent, as the products have been created, they are working well and many companies are interested in them. We have performed the first bacterial tests on several listed bacterial cultures samples collected in Woluwé (Dr Gala). He has demonstrated that the chip is working very well, and that it is specific and sensitive. Would it be necessary to mention this?

Sorry for all the work I cause. You will note that I have done some serious effort to prepare your work. Although my capacities are not comparable to yours, I am gradually learning.

Kind regards,

[signature]

José REMACLE

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Mr Frédéric Luizi
Responsable
Valorisation de la recherche
FUNDP

Namur, le 24 février 2000

Cher Frédéric,

Nous voudrions déposé un brevet sur les nouvelles découvertes que nous avons faites sur les biochips dans le cadre du projet Région Wallone N° 9713634. Ce brevet concerne l'utilisation des biochips ou arrays pour la détection de séquences dans le domaine du diagnostic. Le titre du brevet est « METHOD AND KIT FOR DETECTION AND/OR QUANTIFICATION OF HOMOLOGOUS NUCLEIC ACID SEQUENCES ON ARRAYS «. Le brevet est en correction chez Mr 2ricVan Malderen qui est notre conseiller en brevet.

En ce qui concerne l'originalité de la découverte, j'ai fait une recherche avec Mr Lambermont de l'office de la propriété industrielle de Bruxelles. Cette recherche s'est faite sur base de mots clés et de domaines d'inventions. Ces mots clés étaient les suivant : Chips ou array, DNA ou probe, Capture probe length, homologous sequences. Le domaine de l'invention est le C12 Q1 / 68. Je joins également une copie de ces documents de recherche. Nous n'avons rien trouvé qui correspond à l'invention à savoir une détection de séquences homologues amplifiées et captées sur des chips par des sondes capteurs simples brins fixées sur un support solide et de longueurs comprises entre 30 et 600 bases dont une partie comprise entre 10 et 60 bases est spécifique de la séquence cible à detecter et permettant de détecter des séquences homologues avec une grande sensibilité mais en gardant la spécificité de la détection. L'invention apparaît comme originale et elle donne en tout cas des résultats remarquables. L'orginalité par rapport aux arrays utilisés actuellement qui utilisent des sondes capteur soit très petites soit très grandes, est explicitée dans l'introduction du brevet et je ne la reprends donc pas dans cette lettre. Ce brevet complète le brevet précédent (EU dépôt 99870226.0). Il propose une stratégie alternative qui permet d'éviter le recourt à la PCR multiplex. Dans ce cas-ci une seule paire de primers sont utilisés pour l'amplification

des bactéries apparttenant à une mêm espèce et la discrimination soit de l'espèce , soit de la famille se fait sur le damier.

En ce qui concerne l'intérêt commerciale de l'invention, elle permet d'utiliser les arrays dans le domaine du diagnostic. En effet, la détection et l'identification d'espèces bactériennes par exemple néccessite de pouvoir faire la distinction entre des séquences très proches, c'est à dire homologues. On va donc pouvoir grâce à cette nouvelle approche détecter simultanément sur un array diverses séquences et avoir en un seul test une multitude d'informations. La première application qui est décrite dans ce brevet est la détection des diverses espèces de Staphylocoques et de leur gène de résistance aux antibiotiques. Il s'agit d'un marché immense car nous croyons que d'ici quelques années, cette technologie de détection moléculaire des bactéries sur chips pourra remplacer les tests actuels de microbiologie. Nous pensons à d'autres tests aussi bien bactériens que viraux qui pourraient se faire avec cette même technologie. Par rapport aux tests des chips actuels, ceux qui utilisent de petits trappeurs peuvent faire la discrimination de séquences homologues mais la sensibilité est fortment réduite du fait de la faible vitesse de réaction d'hybridation sur le support comparé à la vitesse en solution. En ce qui concerne les grands trappeurs, ceux-ci ne peuvent pas discréminer les séquences homologues. Ces divers points sont aussi repris dans le brevet et des exemples sont donnés pour conforter ces conclusions.

Etant donné ces renseignements et surtout l'intérêt de cette invention, j'espère que tu pourras transmettre rapisement cette demande à la DGTRE afin de demander l'intervention des fonds attribués par la Région Wallone pour soutenir les dépôts de brevets.

Dès que le brevet est corrigé , je te ferai parvenir une copie. Ceci devrait être fait dans les jours qui viennent étant donné l'urgence de protéger ces résultats et la compétition qu'il y a actuellement dans ce domaine.

Sincèrement

José Remacle

Attn: Mr Frédéric LUIZI
Director Research Valorisation
FUNDP

Namur, February 14, 2000

Dear Frédéric,

We would like to file a patent upon a new discoveries we did conceive relating to biochips within the context of Walloon Region project n° 9713634. This patent concerns the use of biochips or arrays for the detection of sequences in the field of diagnostics. The title of the patent would be "METHOD AND KIT FOR DETECTION AND/OR QUANTIFICATION OF HOMOLOGOUS NUCLEIC ACID SEQUENCES ON ARRAYS". The patent is being corrected by Mr Van Malderen, our patent attorney.

As to the originality of the discovery, I have performed a search in collaboration with Mr Berlamont (i.e. Mr Lambermont) of the Brussels Industrial Property Department. This search was based on key words and on fields of invention. The key words being the following: array, length, capture, probe, homologous. The field of the invention is C12Q1/68. Enclosed, I provide you with a copy of the identified documents. We have not identified anything that corresponds to the invention, i.e. a protection of single strand capture probes fixed upon a solid support and having a length comprised between 30 and 600 base with a part comprised between 10 and 60 base, which is specific of a target sequence to be detected and allowing the detection of homologous sequences with a high sensitivity while maintaining specificity of detection. It is therefore evident that the main claim obviously is too broad and that, during the prosecution, we will have to restrict the scope as presented in the subsidiary claims, which does, however, not reduce the originality of the invention. This originality, compared to arrays currently used that requires the capture probes either very small or very long, as explicitly mentioned in the introduction of the present patent, which I did hence not refer to in this letter. This patent completes the previous patent (EU filing 99870226.0). It proposes an alternative strategy, which allows avoiding the use of multiplex PCR. In the present case, only a single pair of primers is used for the amplification, and the discrimination is obtained upon the array.

The commercial interest of the invention is that it allows using arrays in the field of diagnostics. Indeed, the detection and identification of, for instance, bacterial species requires being able to distinct between very close, i.e. homologous, sequences. This new method will thus allow us to simultaneously detect several sequences on an array, and to acquire multiple data by a single test. The first application described in this patent is the detection of several staphylococcus species and their genes that resist antibiotics. A huge market is concerned here, as we believe that, during the following years, such molecular

detection technology for bacteria upon chips might replace the present microbiological tests. We do believe other tests relating to bacteria or viruses could be performed using the same technology. Compared to the present chips tests, the ones using a small capture probe can discriminate homologous sequences, the sensibility, however, is heavily reduced due to the slow speed of hybridization reaction on the support compared to the speed in a solution. As to long capture probes, these are not able to discriminate homologous sequences. The various arguments given are included in the patent and examples are provided to support the above comments.

In view of the above comments and of the invention's advantages, I do hope that you will be able to accept the filing and call for the funds obtained from the Walloon Region for supporting patent filings.

Sincerely,

[signature]

José REMACLE

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Frédéric Luizi
Services des Relations Extérieures

Namur, le 29 février 2000

Cher Frédéric,

Objet : Brevet METHOD AND KIT FOR DETECTION AND/OR QUANTIFICATION OF HOMOLOGOUS NUCLEIC ACID SEQUENCES ON ARRAYS

En ce qui concerne l'urgence du brevet, celle-ci s'impose du fait que les résultats que nous avons obtenus en utilisant cette technologie a permis de détecter les Staphylocoques grâce à une biochip. Celle-ci va être utilisée dans les 2 semaines qui viennent à Woluwe et nous comptons la montrer à d'autres laboratoires intéressés. Nous aimerions donc que le brevet soit déposé avant que l'on puisse la montrer.

D'autre part, dans le domaine des biochips, beaucoup de sociétés sont en train de prendre des brevets et le domaine est extrêmement compétitif surtout en ce qui concerne les applications comme celles que nous proposons dans ce brevet c'est-à-dire le diagnostic médical. Un retard de quelques mois serait catastrophique.

J'espère que tu vas pouvoir accepter ce dépôt. En te remerciant,

José Remacle

Letter to Frédéric LUIZI
Service des Relations Extérieures
Namur

February 29, 2000

Dear Frédéric,

Object : patent - method and kit for detection and/or quantification of homologous nucleic acid sequences on arrays

The urgency of the patent is due to the fact that the results we have obtained by using this technology have allowed us to detect the Staphylocoques by the biochips.

These biochips will be used in the two following weeks in Woluwé and we propose to show them to other interested laboratories.

We hope that the patent will be filed before we can show it.

Furthermore, in the field of biochips, a lot of companies are patenting and the field is extremely competitive, when it is related to applications, such the ones we propose in this patent, especially medical diagnosis. A delay of several months would be catastrophic.

I hope that you can accelerate this filing.

Thank you.

José Remacle.